

48

1652

Y. Pak
 RAW SEQUENCE LISTING DATE: 08/01/2001
 PATENT APPLICATION: US/09/654,652A TIME: 13:55:40

Input Set : A:\4910-8.app
 Output Set: N:\CRF3\08012001\I654652A.raw

3 <110> APPLICANT: SHYUR, LIE-FEN
 4 CHEN, JUI-LIN
 5 YANG, NING-SUN
 7 <120> TITLE OF INVENTION: A TRUNCATED FORM OF FIBROBACTER SUCCINOGENES 1, 3-1,
 8 4-BETA-D-GLUCANASE WITH IMPROVED ENZYMATIC ACTIVITY AND
 9 THERMO-TOLERANCE
 11 <130> FILE REFERENCE: 4910-8
 13 <140> CURRENT APPLICATION NUMBER: 09/654,652A
 14 <141> CURRENT FILING DATE: 2000-09-05
 16 <160> NUMBER OF SEQ ID NOS: 11
 18 <170> SOFTWARE: PatentIn Ver. 2.1
 20 <210> SEQ ID NO: 1
 21 <211> LENGTH: 248
 22 <212> TYPE: PRT
 23 <213> ORGANISM: Artificial Sequence
 25 <220> FEATURE:
 26 <223> OTHER INFORMATION: Description of Artificial Sequence: Modified enzyme
 27 with enhanced activity and thermal stability
 29 <400> SEQUENCE: 1
 30 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
 31 1 5 10 15
 33 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
 34 20 25 30
 36 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
 37 35 40 45
 39 Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
 40 50 55 60
 42 Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
 43 65 70 75 80
 45 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
 46 85 90 95
 48 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
 49 100 105 110
 51 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
 52 115 120 125
 54 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
 55 130 135 140
 57 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
 58 145 150 155 160
 60 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
 61 165 170 175
 63 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
 64 180 185 190
 66 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
 67 195 200 205
 69 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu
 70 210 215 220

ENTERED

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72 Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val
73 225 230 235 240
75 Pro Arg Asp Asp Glu Pro Ala Pro
76 245
79 <210> SEQ ID NO: 2
80 <211> LENGTH: 267
81 <212> TYPE: PRT
82 <213> ORGANISM: Artificial Sequence
84 <220> FEATURE:
85 <223> OTHER INFORMATION: Description of Artificial Sequence: Modified enzyme
86 with enhanced activity and thermal stability
88 <400> SEQUENCE: 2
89 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
90 1 5 10 15
92 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
93 20 25 30
95 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
96 35 40 45
98 Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
99 50 55 60
101 Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
102 65 70 75 80
104 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
105 85 90 95
107 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
108 100 105 110
110 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gln Val Ser
111 115 120 125
113 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
114 130 135 140
116 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
117 145 150 155 160
119 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
120 165 170 175
122 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
123 180 185 190
125 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
126 195 200 205
128 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu
129 210 215 220
131 Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val
132 225 230 235 240
134 Pro Arg Asp Asp Glu Pro Ala Pro Asn Ser Ser Ser Val Asp Lys Leu
135 245 250 255
137 Ala Ala Ala Leu Glu His His His His His His
138 260 265
141 <210> SEQ ID NO: 3
142 <211> LENGTH: 349
143 <212> TYPE: PRT

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144 <213> ORGANISM: Fibrobacter succinogenes
 146 <400> SEQUENCE: 3
 147 Met Asn Ile Lys Lys Thr Ala Val Lys Ser Ala Leu Ala Val Ala Ala
 148 1 5 10 15
 150 Ala Ala Ala Ala Leu Thr Thr Asn Val Ser Ala Lys Asp Phe Ser Gly
 151 20 25 30
 153 Ala Glu Leu Tyr Thr Leu Glu Glu Val Gln Tyr Gly Lys Phe Glu Ala
 154 35 40 45
 156 Arg Met Lys Met Ala Ala Ala Ser Gly Thr Val Ser Ser Met Phe Leu
 157 50 55 60
 159 Tyr Gln Asn Gly Ser Glu Ile Ala Asp Gly Arg Pro Trp Val Glu Val
 160 65 70 75 80
 162 Asp Ile Glu Val Leu Gly Lys Asn Pro Gly Ser Phe Gln Ser Asn Ile
 163 85 90 95
 165 Ile Thr Gly Lys Ala Gly Ala Gln Lys Thr Ser Glu Lys His His Ala
 166 100 105 110
 168 Val Ser Pro Ala Ala Asp Gln Ala Phe His Thr Tyr Gly Leu Glu Trp
 169 115 120 125
 171 Thr Pro Asn Tyr Val Arg Trp Thr Val Asp Gly Gln Glu Val Arg Lys
 172 130 135 140
 174 Thr Glu Gly Gly Gln Val Ser Asn Leu Thr Gly Thr Gln Gly Leu Arg
 175 145 150 155 160
 177 Phe Asn Leu Trp Ser Ser Glu Ser Ala Ala Trp Val Gly Gln Phe Asp
 178 165 170 175
 180 Glu Ser Lys Leu Pro Leu Phe Gln Phe Ile Asn Trp Val Lys Val Tyr
 181 180 185 190
 183 Lys Tyr Thr Pro Gly Gln Gly Glu Gly Ser Asp Phe Thr Leu Asp
 184 195 200 205
 186 Trp Thr Asp Asn Phe Asp Thr Phe Asp Gly Ser Arg Trp Gly Lys Gly
 187 210 215 220
 189 Asp Trp Thr Phe Asp Gly Asn Arg Val Asp Leu Thr Asp Lys Asn Ile
 190 225 230 235 240
 192 Tyr Ser Arg Asp Gly Met Leu Ile Leu Ala Leu Thr Arg Lys Gly Gln
 193 245 250 255
 195 Glu Ser Phe Asn Gly Gln Val Pro Arg Asp Asp Glu Pro Ala Pro Gln
 196 260 265 270
 198 Ser Ser Ser Ala Pro Ala Ser Ser Ser Val Pro Ala Ser Ser
 199 275 280 285
 201 Ser Ser Val Pro Ala Ser Ser Ser Ala Phe Val Pro Pro Ser Ser
 202 290 295 300
 204 Ser Ser Ala Thr Asn Ala Ile His Gly Met Arg Thr Thr Pro Ala Val
 205 305 310 315 320
 207 Ala Lys Glu His Arg Asn Leu Val Asn Ala Lys Gly Ala Lys Val Asn
 208 325 330 335
 210 Pro Asn Gly His Lys Arg Tyr Arg Val Asn Phe Glu His
 211 340 345
 214 <210> SEQ ID NO: 4
 215 <211> LENGTH: 744
 216 <212> TYPE: DNA

RAW SEQUENCE LISTING
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217 <213> ORGANISM: Artificial Sequence
 219 <220> FEATURE:
 220 <223> OTHER INFORMATION: Description of Artificial Sequence: DNA encoding a
 221 modified enzyme
 223 <220> FEATURE:
 224 <221> NAME/KEY: CDS
 225 <222> LOCATION: (1)..(744)
 227 <400> SEQUENCE: 4
 228 atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa 48
 229 Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu
 230 1 5 10 15
 232 gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca 96
 233 Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala
 234 20 25 30
 236 tcg gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc 144
 237 Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile
 238 35 40 45
 240 gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag 192
 241 Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys
 242 50 55 60
 244 aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca 240
 245 Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala
 246 65 70 75 80
 248 caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gcc gat cag 288
 249 Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln
 250 85 90 95
 252 gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg 336
 253 Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp
 254 100 105 110
 256 act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc 384
 257 Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser
 258 115 120 125
 260 aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag 432
 261 Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu
 262 130 135 140
 264 agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc 480
 265 Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe
 266 145 150 155 160
 268 cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc 528
 269 Gln Phe Ile Asn Trp Val Lys Val Tyr Lys Tyr Thr Pro Gly Gln Gly
 270 165 170 175
 272 gaa ggc ggc agc gac ttt acg ctt gac tgg acc gac aat ttt gac acg 576
 273 Glu Gly Gly Ser Asp Phe Thr Leu Asp Trp Thr Asp Asn Phe Asp Thr
 274 180 185 190
 276 ttt gat ggc tcc cgc tgg ggc aag ggt gac tgg aca ttt gac ggt aac 624
 277 Phe Asp Gly Ser Arg Trp Gly Lys Gly Asp Trp Thr Phe Asp Gly Asn
 278 195 200 205
 280 cgt gtc gac ctc acc gac aag aac atc tac tcc aga gat ggc atg ttg 672
 281 Arg Val Asp Leu Thr Asp Lys Asn Ile Tyr Ser Arg Asp Gly Met Leu

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282	210	215	220	
284	atc ctc gcc ctc acc cgc aaa ggt cag gaa agc ttc aac ggc cag gtt			720
285	Ile Leu Ala Leu Thr Arg Lys Gly Gln Glu Ser Phe Asn Gly Gln Val			
286	225	230	235	240
288	ccg aga gat gac gaa cct gct ccg			744
289	Pro Arg Asp Asp Glu Pro Ala Pro			
290	245			
293	<210> SEQ ID NO: 5			
294	<211> LENGTH: 804			
295	<212> TYPE: DNA			
296	<213> ORGANISM: Artificial Sequence			
298	<220> FEATURE:			
299	<223> OTHER INFORMATION: Description of Artificial Sequence: DNA encoding a			
300	modified enzyme			
302	<220> FEATURE:			
303	<221> NAME/KEY: CDS			
304	<222> LOCATION: (1)..(804)			
306	<400> SEQUENCE: 5			
307	atg gtt agc gca aag gat ttt agc ggt gcc gaa ctc tac acg tta gaa			48
308	Met Val Ser Ala Lys Asp Phe Ser Gly Ala Glu Leu Tyr Thr Leu Glu			
309	1 5 10 15			
311	gaa gtt cag tac ggt aag ttt gaa gcc cgt atg aag atg gca gcc gca			96
312	Glu Val Gln Tyr Gly Lys Phe Glu Ala Arg Met Lys Met Ala Ala Ala			
313	20 25 30			
315	tgc gga aca gtc agt tcc atg ttc ctc tac cag aat ggt tcc gaa atc			144
316	Ser Gly Thr Val Ser Ser Met Phe Leu Tyr Gln Asn Gly Ser Glu Ile			
317	35 40 45			
319	gcc gat gga agg ccc tgg gta gaa gtg gat att gaa gtt ctc ggc aag			192
320	Ala Asp Gly Arg Pro Trp Val Glu Val Asp Ile Glu Val Leu Gly Lys			
321	50 55 60			
323	aat ccg ggc agt ttc cag tcc aac atc att acc ggt aag gcc ggc gca			240
324	Asn Pro Gly Ser Phe Gln Ser Asn Ile Ile Thr Gly Lys Ala Gly Ala			
325	65 70 75 80			
327	caa aag act agc gaa aag cac cat gct gtt agc ccc gcc gca gat cag			288
328	Gln Lys Thr Ser Glu Lys His His Ala Val Ser Pro Ala Ala Asp Gln			
329	85 90 95			
331	gct ttc cac acc tac ggt ctc gaa tgg act ccg aat tac gtc cgc tgg			336
332	Ala Phe His Thr Tyr Gly Leu Glu Trp Thr Pro Asn Tyr Val Arg Trp			
333	100 105 110			
335	act gtt gac ggt cag gaa gtc cgc aag acg gaa ggt ggc cag gtt tcc			384
336	Thr Val Asp Gly Gln Glu Val Arg Lys Thr Glu Gly Gly Gln Val Ser			
337	115 120 125			
339	aac ttg aca ggt aca cag gga ctc cgt ttt aac ctt tgg tcg tct gag			432
340	Asn Leu Thr Gly Thr Gln Gly Leu Arg Phe Asn Leu Trp Ser Ser Glu			
341	130 135 140			
343	agt gcg gct tgg gtt ggc cag ttc gat gaa tca aag ctt ccg ctt ttc			480
344	Ser Ala Ala Trp Val Gly Gln Phe Asp Glu Ser Lys Leu Pro Leu Phe			
345	145 150 155 160			
347	cag ttc atc aac tgg gtc aag gtt tat aag tat acg ccg ggc cag ggc			528

→ Use of n and/or Xaa has been detected in the Sequence Listing.
Review the Sequence Listing to insure a corresponding explanation is presented in the <220> to <223> fields of each sequence using n or Xaa.

VERIFICATION SUMMARY

PATENT APPLICATION: US/09/654,652A

DATE: 08/01/2001

TIME: 13:55:41

Input Set : A:\4910-8.app

Output Set: N:\CRF3\08012001\I654652A.raw

L:539 M:341 W: (46) "n" or "Xaa" used, for SEQ ID#:11